REMARKS

Applicants respectfully request allowance of this application in view of the amendments above and the following comments.

The Communication dated May 13, 2002, required Applicants to amend the specification to identify the sequences as to "SEQ ID NO". This has now been done. Also, with respect to Figure 2, Applicants have followed the Examiner's suggestion and identified the corresponding SEQ ID NOs on page 18 of the specification, as opposed to on Figure 2 itself. A clean copy of the amended portions of the specification appears above, and a mark-up showing the changes made is attached. Applicants do not believe that any new matter has been introduced.

Early and favorable action is earnestly solicited.

Respectfully submitted.

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By

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CERTIFICATE OF MAILING

I hereby certify that the foregoing Preliminary Amendment and the attached Mark-Up Showing the Changes Made in the Specification to Yield the Specification as Amended Above are being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on the date indicated below:

Date: August 13, 2002 By: Kurt G. Briscoe

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MARK-UP SHOWING THE CHANGES MADE IN THE SPECIFICATION TO YIELD THE SPECIFICATION AS AMENDED ABOVE

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Page 18, last full paragraph:

Figure 2: Comparison of the DNA sequences of the lipase mutants P1B 01-H1 **(SEQ ID NO: 3)**, P1B 01-E4 **(SEQ ID NO: 5)**, P2B 08-H3 **(SEQ ID NO: 7)**, P3B 13-D10 **(SEQ ID NO: 11)**, P4B 04-H3 **(SEQ ID NO: 13)**, P5B 14-C11 **(SEQ ID NO: 15)** and P4BSF 03-G10 S155F **(SEQ ID NO: 17)** with the sequence of the wild type **(SEQ ID NO: 9)** of lipase from *P. aeruginosa* (the mutated bases with respect to the wild type are boxed, the origin of the mature lipase mutants is at base 163 or at base 162 in the wild type.)

Page 21, third paragraph:

The lipase gene *lipA* is amplified using the plasmid pMut5 linearized with endonuclease *Xmn* I as a template and the following PCR primers:

A: 5'-GCGCAATTAACCCTCACTAAAGGGAACAAA-3' **(SEQ ID NO: 1)**

B: 5'-GCGTAATACGACTCACTATAGGGCGAA-3' **(SEQ ID NO: 2)**

Page 29, last paragraph:

By sequencing the positive mutants, the mutations could be localized within the lipase genes (see Figure 2). After assigning the base triplets to the corresponding amino acids, the following amino acid substitutions result with respect to the wild type lipase from *P. aeruginosa*:

P1B 01-H1: T103I (Thr103 → Ile103), S149G (Ser149 → Gly149) **(amino acid sequence shown in SEQ ID NO: 4)**

P1B 01-E4: S149G (Ser149 → Gly149) **(amino acid sequence shown in SEQ ID NO: 6)**

P2B 08-H3: S149G (Ser149 → Gly149), S155L (Ser155 → Leu155) **(amino acid sequence shown in SEQ ID NO: 8)**

P3B 13-D10: S149G (Ser 149 → Gly149), S155L (Ser 155 → Leu155), V47G (Val47 → Gly47) **(amino acid sequence shown in SEQ ID NO: 12)**

P4B 04-H3: S149G (Ser149 → Gly149), S155L (Ser155 → Leu155), V47G (Val47 → Gly47), S33N (Ser33 → Asn33), F259L (Phe259 → Leu259) **(amino acid sequence shown in SEQ ID NO: 14**

P5B 14-C11: S149G (Ser149 → Gly149), S155L (Ser155 → Leu155), V47G (Val47 → Gly47), S33N (Ser33 → Asn33), F259L (Phe259 → Leu259), K110R (Lys110 → Arg110) **(amino acid sequence shown in SEQ ID NO: 16)**

Page 33, last paragraph:

A fragment of the gene for the lipase from mutant P3B 13D10 is amplified using plasmid pMut5 13 D10, linearized by endonuclease XmnI, and the following PCR primers:

A: 5'-GCGCAATTAACCCTCACTAAAGGGAACAAA-3' **(SEQ ID NO: 19)**

M: 5'-GGTACGCAGAATNNNCTGGGCTCGC-3' **(SEQ ID NO: 20)**

Page 34, first full paragraph:

The reaction conditions are as follows: A 50 µl reaction volume contains 75mM Tris/HCl, pH

9.0 (at 25°C); 20 mM $(NH_4)_2SO_4$; 1.5 mM $MgCl_2$; 0.01% (w/v) Tween® 20; 10% (v/v) DMSO; 10 pmol of each of the primers; 0.1 ng of the template DNA; and 2 U of Taq polymerase (Goldstar, Eurogentec). The cycling protocol is as follows: A 2 min denaturation at 98°C is followed by 30 cycles with 1 min at 94°C, 2 min at 64°C, 1 min at 72°C on a Robocycler 40 (Stratagene), followed by incubation for 7 min at 72°C. The Taq polymerase is added after the denaturation of the 1st cycle. After purification of the PCR products by agarose gel electrophoresis and elution of the DNA from the agarose gel using the Nucleospin Extract Kit (Macherey & Nagel), it was used as a primer (so-called megaprimer) in a subsequent PCR. Thus, the lipase gene is amplified on the plasmid pMut5ΔAK 13D10, linearized by endonuclease XmnI, using the following PCR primers and the above described reaction conditions:

A: 5'-GCGCAATTAACCCTCACTAAAGGGAACAAA-3' **(SEQ ID NO: 19)**

B (megaprimer): 5'-GCGTAATACGACTCACTATAGGGCGAA-3' **(SEQ ID NO: 21)**

Page 36, first paragraph:

By sequencing the positive mutants, the mutations could be localized within the lipase gene (see Figure 2). After assigning the base triplets to the corresponding amino acids, the following amino acid substitution resulted with respect to mutant P3B 13-D10:

P4BSF 03-G10: L155F (Leu155 → Phe155) **(amino acid sequence shown in SEQ ID NO: 18)**